Summary of Office Action

Examination of claims 5 and 9-14 is reported in the present Office Action. Claims 5 and 9-14 are rejected under 35 U.S.C. § 112, first paragraph. The rejection is addressed below.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 5 and 9-14 stand rejected as lacking enablement. The claims feature methods involving the *in vivo* administration of XIAP antisense nucleic acids to induce apoptosis in a cell. The Examiner asserts that the specification fails to enable the *in vivo* therapeutic use of antisense nucleic acid molecules complementary to human XIAP. This rejection is respectfully traversed.

The Examiner's rejection hinges on the assertion that delivering antisense oligonucleotides presents an obstacle to their therapeutic use because existing methods for the delivery of oligonucleotides are inadequate. To support the enablement rejection the Examiner cites Chirila et al., (Biomaterials 23:321-342, 2002, hereafter "Chirila"), Jen et al. (Stem Cells 18:307-319, 2000, hereafter "Jen"), and Stein (Pharmacology & Therapeutics 85:231-236, hereafter "Stein").

Chirila

Chirila provides a review of the use of synthetic polymers for delivery of therapeutic antisense oligonucleotides.

The Examiner cites passages from Chirila to support the assertion that methods for the *in vivo* delivery of oligonucleotides were inadequate at the time of filing, October 9, 2001. For example, the Examiner asserts that antisense oligonucleotide delivery must be maintained for a sufficient period of time to achieve therapeutic efficacy and that at the time of filing methods for such delivery were inadequate. A thorough reading of the entire passage suggests that this challenge can be overcome by repeated administration, a method known to the skilled artisan at the time of filing. At page 327, right column, last paragraph, Chirila states, "Many studies indicate that although ODNs can gain access to

the target tissue *in vivo*, they are eliminated rapidly and repeated administration is required to achieve therapeutic effects [9] (emphasis added)."

In another passage cited by the Examiner, Chirila appears to support the assertion that methods of antisense delivery at the time of filing were insufficient. A thorough reading of the entire passage suggests that it may be *the use of polymers* as a delivery system that holds future promise. Polymer delivery strategies are discussed as a potential replacement for existing oligonucleotide delivery methods. At page 337, left column, lines 26-48, Chirila states:

> In spite of profuse research, none of the polymer carriers developed so far were able to replace convincingly as a delivery system the infusion (or injection) of free or liposome-encapsulated AS ODNs [antisense oligonuclotides], although some of the proposed carriers (cationic polymers, biodegradable polymers) showed promising results...However, the fact that the outcome so far is not of decisive help in establishing which polymers shall be the carriers of choice is rather a moot point. This review clearly demonstrates a consistent and ever increasing interest in the polymer-aided delivery of therapeutic AS ODNs, which brings hopes that this biomaterials application will be successful in the forthcoming future. As a new generation of drug therapy in an advanced stage of development, the antisense strategy only awaits a suitable delivery system in order to live up to its promise.

With respect to existing methods for antisense oligonucleotide delivery, Chirila states:

> There is, however, some skepticism about the need for delivery systems for AS ODNs [15]...Since the AS ODNs can be delivered without any carrier and they still can display AS activity, is there any need to bother with developing delivery systems? In reply, there are many reports indicating enhanced therapeutic effect of AS ODNs when they are delivered in association with an adjuvant (page 327, right column, first paragraph). (Emphasis added.)

Thus, Chirila accepts that antisense nucleic acids delivered by infusion or injection display antisense activity, but advocates the development of alternate delivery systems to enhance the therapeutic efficacy of these antisense oligonucleotides.

In fact, Chirila provides numerous examples demonstrating the successful use of antisense oligonucleotides *in vivo*. At page 322, right column, first paragraph, Chirila observes that a commercial antisense therapeutic, Fomivirsen, was approved by the FDA

as a treatment for cytomegalovirus retinitis; at page 326, left column, lines 6-11, Chirila notes that antisense effects were obtained *in vivo* with naked oligonucleotides in animal and clinical trials; at page 330, right column, second paragraph, Chirila reveals that antisense oligonucleotides, covalently coupled to poly(L-lysine), were effectively delivered to hepatoma cells *in vitro* and *in vivo,* citing Wu et al., (Wu et al., J. Biol. Chem. 263:14621-4; 1988; Wu, J. Biol. Chem. 262:4429-32; 1987; J. Biol. Chem. 267:12436-9, 1992); at page 330, right column, last paragraph,; Chirila notes that the poly(L-lysine) oligonucleotide complexes were used to efficiently deliver antisense oligonucleotides to animal organs where they targeted a retroviral mRNA; at page 332, right column, lines 12-16, Chirila notes that polyspermine poly(ethylene oxide) copolymers were used to deliver antisense oligonucleotides into the vitreous cavity of rat eyes where they successfully downregulated expression of their target, fibronectin, citing Roy et al., (Nature Biotech. 17:476-9, 1999); at page 333, left column, lines 15-22, Chirila relates that antisense oligonucleotides were used to inhibit tumour growth in mice injected with oncogene-carrying cells; at page 335, right column, second paragraph, Chirila, citing Edelman et al., (Circ. Res. 76:176-82, 1995), observes that antisense oligonucleotides in poly(ethylene-co-vinyl acetate) produced a 99.6% inhibition of hyperplasia when surgically implanted around denuded rat carotid artery. Finally, Chirila notes, at page 336, right column, lines 6-11, that antisense oligonucleotides in hydrogels inhibited cell proliferation in pig arteries after angioplasty, citing Azrin et al. (Cath. Cardiovasc. Diag. 41:232-40, 1997).

In sum, Chirila fails to support the Examiner's assertion that at the time of filing methods for the *in vivo* delivery of therapeutic oligonucleotides were unpredictable. In fact, contrary to this assertion, Chirila provides numerous examples demonstrating the successful therapeutic use of antisense oligonucleotides. Thus, this basis for the enablement rejection should be withdrawn.

Jen

Jen provides a review of strategies and options for suppressing gene expression by targeting mRNA. The Examiner cites Jen in support of the assertion that anti-sense therapy methods are not routine. In a passage cited by the Examiner, Jen opines, "the effective and efficient clinical translation of the antisense strategy has remained elusive." Jen supports this opinion by citing Waters et al. (J. Clin. Oncol. 18:1812-1823, 2000). In particular Jen states:

> While a number of phaseI/II trials employing ONs [oligonucleotides] have been reported, virtually all have been characterized by a lack of toxicity but only modest clinical effects. A recent paper by Waters et al. describing the use of a bcl-2-targeted ON in patients with non-Hodgkin's lymphoma is typical in this regard (page 315, lines 9-15).

While Jen characterizes Water's results as showing only "modest clinical effects," Waters et al.'s *in vivo* administration of antisense oligonucleotides down regulated the target protein in many of the patients that received treatment (Waters et al., J. Clin. Oncol. 18:1812-1823, 2000, Exhibit A,). This is particularly impressive given that Waters et al. describe a phase I trial, designed primarily to assess toxicity, rather than efficacy. On this point, Waters et al. state:

> Bcl-2 protein was reduced in seven of 16 assessable patients. This reduction occurred in tumor cells derived from lymph nodes in two patients and from peripheral blood or bone marrow mononuclear cell populations in the remaining five patients.
>
> CONCLUSION: Bcl-2 antisense therapy is feasible and shows potential for antitumor activity in NHL [Non-Hodgkin's Lymphoma]. Downregulation of Bcl-2 protein suggests a specific antisense mechanism (emphasis added) (page 1812, right column, abstract).

Waters et al. successfully used antisense oliognucleotides *in vivo* to inhibit the biological activity of a target protein. This is no more than applicants' claims require. Thus, Waters et al. fail to support Jen's opinions regarding the difficulties associated with the therapeutic use of antisense oligonucleotides *in vivo*.

Stein

The Examiner cites Stein to support the assertion that the use of antisense oligonucleotide therapeutics is unpredictable because such use lacks sequence specificity. In fact, a thorough reading of Stein fails to support this assertion. While Stein believes that nonsequence specific effects are a problem for researchers interested in determining the function of particular genes, Stein does not believe that such effects are a problem for medical providers interested in the therapeutic use of antisense oligonucleotides. On this point, Stein states:

> These nonsequence-specific effects of phosphorothioate oligonucleotides themselves may be therapeutic. Sorting out specific and nonspecific mechanisms of action may often be extremely difficult, if not well nigh impossible. Fortunately, this matters neither to the physical health of a patient being treated with an antisense oligonucleotide nor to the financial health of the treating entity (page 232, left column, paragraph spanning pages 231 and 232).

In fact, Stein provides examples of the successful therapeutic use of the non-sequence specific antisense therapeutic Fomivirsen.

> In this particular case, however, while Fomivirsen is undeniably active clinically, some doubts remain as to its mechanism of action. These doubts relate to the nonspecificity of phosphorothioates, engendered by their ability to nonsequence-specifically bind heparin-binding proteins (page 231, right column, paragraph spanning pages 231 and 232).

In fact, regarding the use of antisense oligonucleotides, Stein states, "Over the past decade, the antisense biotechnology has been employed many times to reproducibly demonstrate truly stunning down-regulation in a variety of systems (page 231, left column, first paragraph). While Stein acknowledges that conflicts exist regarding some data, Stein regards the antisense data as widely reproducible.

> ...[T]argets, such as protein kinase C (PKC)-α, c-raf kinase, intercellular adhesion molecule-1, and bcl-2 have been examined by several laboratories, producing a consensus that in these examples down-regulation of translation is indeed due to a Watson-Crick hybridization mechanism. Indeed, evidence exists that such a mechanism may have clinical applicability (page 231, left column, first paragraph, continued in the right column).

Stein goes on to cite examples of the successful therapeutic use of anti-sense oligonucleotides *in vivo*, in particular, the use of phosphorothioate antisense oligonucleotides to target intercellular adhesion molecule-1 mRNA for the treatment of Crohn's disease (page 231, left column, first paragraph, continued in the right column) and the use of Fomivirsen, a phosphorothioate oligodeoxynucleotide, to target cytomegalovirus immediate early mRNA as a treatment for cytomegalovirus retinitis (page 231, right column, first paragraph, continued on page 232).

In sum, Chirila, Stein, and Jen fail to support the Examiner's assertion that methods for delivering antisense oligonucleotides provided an obstacle to their therapeutic use at the time of filing. In fact, Chirila and Stein tend to support applicants' position that at the time of filing antisense oligonucleotides could predictably target specific mRNA sequences for inhibition.

Undue Experimentation

The standard for determining whether a specification enables a claimed invention was set forth in *Mineral Separation v Hyde*, 242 U.S. 261, 270 (1916). In this case, the Supreme Court posed the question: is the experimentation needed to practice the invention undue or unreasonable? *United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQd at 1404 (Fed. Cir. 1988) "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation."

The state of the art at the time of filing suggests that antisense oligonucleotides were used predictably to target and down regulate *in vivo* mRNAs in a variety of systems. In fact, Stein and Chirila provide numerous examples of the successful *in vivo* therapeutic use of antisense oligonucleotides.

Guidelines for in vivo antisense delivery

Contrary to the Examiner's assertions, applicants provide detailed methods that allow the skilled artisan to predictably practice the methods of the invention. For example, applicants disclose methods for the identification of antisense oligonucleotides (page 54, lines 10-21); methods for the rapid screening of IAP antisense therapies in cell lines (page 25, lines 10-22, and page 55, lines 8-18); methods for optimization of antisense therapies (page 55, lines 3-7), and methods for the *in vivo* delivery of therapeutic antisense oligonucleotides, including viral (pages 26 and 27), non-viral (page 27, line 21, to page 28), and injection methods (page 27, lines 5-9). In addition, applicants provide methods to test the efficacy of identified antisense oligonucleotides in animal models (pages 55 and 56), either alone or in combination with traditional therapies. A skilled artisan provided with applicants disclosure of XIAP sequences and using no more than routine methods could practice the full scope of the invention as claimed.

In vivo efficacy of antisense therapy reduced to practice

The sufficiency of applicants disclosure is evidenced in the Declaration of Dr. Robert Korneluk, submitted herewith, showing that applicants have successfully reduced to practice the present invention using techniques known to those skilled in the art of antisense oligonucleotide technology at the time of filing. Specifically, applicants i) identified antisense oligonucleotides; ii) rapidly screened IAP antisense therapies in cell lines; iii) tested the efficacy of identified antisense oligonucleotides in animal models; and iv) tested the efficacy of antisense oligonucleotides in combination with traditional therapies. These experiments, as detailed below, plainly demonstrate that the *in vivo* administration of antisense oligonucleotides complementary to XIAP SEQ ID NO:3 was enabled as a cancer therapy at the time applicants' priority document was filed.

Identification of antisense oligonucleotides

In the Declaration of Dr. Robert Korneluk, paragraph 4, applicants disclose that antisense oligonucleotides were identified using methods outlined in our disclosure. Specifically, applicants identified ninety-six oligonucleotide sequences complementary to a portion of XIAP (each sequence having nineteen nucleotides) (SEQ ID NOs: 1 through 96; Table 1) (Exhibit A), from a region approximately 1 kb upstream of the start codon to approximately 1 kb downstream of the stop codon of the XIAP cDNA sequence.

In vitro screening of oligonucleotides

At paragraph 5, applicants disclose that the XIAP synthetic library of 96 antisense oligonucleotides was rapidly screened in cell lines. Specifically, T24 cells (1.5×10^4 cells/well) were seeded in wells of a 96-well plate on day 1, and were cultured in antibiotic-free McCoy's medium for 24 hours. On day 2, the cells were transfected with XIAP antisense oligonucleotides. On day 3, XIAP RNA levels were measured using quantitative real-time PCR techniques. At day 4, XIAP protein levels were measured by ELISA (Exhibit B, Figs. 1A, 1C, 1E, 1G, 1I, and 1K) and total cellular protein was measured biochemically (Exhibit B, Figs. 1B, 1D, 1F, 1H, 1J, and 1L); and used to normalize the XIAP protein levels). These results were compared to a mock transfection sample (treated with the transfection agent without oligonucleotide DNA, and then processed as for the other samples).

These methods identified 16 antisense oligonucleotides that decreased XIAP protein levels relative to control cells that were mock transfected. As expected, the ability of an antisense oligonucleotide to decrease XIAP protein levels correlated with its ability to decrease XIAP mRNA levels (Exhibit C). Sixteen of these oligonucleotides decreased by at least 50% levels of XIAP protein or mRNA levels. The G4 antisense oligonucleotides (G4AS) exhibited the strongest down-regulating effect on XIAP protein, reducing XIAP protein levels by 62% within twenty-four hours after the end of transfection (Exhibit D) when compared with control (CNT), lipofectin alone (LFA), other antisense oligonucleotides (F3AS, C5AS), and oligonucleotide controls (F3RP,

G4SC, C5RP). As shown in Exhibit E-A, cells treated with G4 antisense oligonucleotides had higher levels of apoptotic DNA content than control cells or cells treated with a G4 control oligonucleotide, and underwent morphological changes characteristic of apoptosis Exhibit E-B). Few control cells showed these morphological changes. Forty-eight hours after transfection, G4 antisense oligonucleotides reduced H460 cell growth 55% relative to untreated controls (Exhibit F). *In vivo* studies with lung carcinoma and breast carcinoma cells were then carried out.

Efficacy testing in animal models

In paragraphs 6 and 7, applicants disclose that the efficacy of identified antisense oligonucleotides was tested in animal models. In particular, applicants teach that SCID-RAG2 mice were inoculated with H460 human lung carcinoma cells (subcutaneous shoulder injection of 10^6 cells) and treatments with G4 and F3 AS PS-ODNs, as well as a scrambled control, were initiated three days after tumor inoculation. Oligonucleotide injections were administered intraperitoneally at 12.5 mg/kg three times a week for three weeks. At the end of the treatment period, mean tumor sizes in the groups treated with either G4 or F3 antisense oligonucleotides were ~ 50 % smaller relative to tumor size in a control group treated with a scrambled control oligonucleotide (C5 scr) or vehicle alone (Veh. Co) (Exhibit G).

The treatment protocol described above was also tested on female SCID-RAG2 mice in groups 1-4 (GP1-4) inoculated orthotopically with MDA-MB-435/LCC6 human breast carcinoma cells. Two weeks after the last treatment (day 35) tumor volumes of mice treated with F3, C5 or G4 antisense oligonucleotides were 70 %, 60 % and 45 % smaller than vehicle controls (GP1:Veh. Co) (Exhibit H).

At paragraph 8, applicants further disclose that G4 antisense oligonucleotides in SCID-RAG2 mice bearing xenografts of H460 human non-small-cell lung tumors were implanted subcutaneously. Saline-treated control tumors (Veh. Co) grew reproducibly to a size of 0.75 cm^3 within approximately 24 days (Exhibit I). Oligonucleotide treatments were initiated three days after tumor cell inoculation. G4 antisense oligonucleotides (5 to

15 mg/kg) or a control oligonucleotide (G4 SM) were administered using a treatment schedule of intraperitoneal injections given on days 3-7, 10-14, and 17-21 (once a day). The treatment with 5 or 15 mg/kg G4 antisense oligonucleotides greatly delayed tumor growth: on day 24 mean tumor sizes were 0.75, 0.45 and 0.29 cm^3 in control, 5 and 15 mg/kg treated groups, respectively (Exhibit I). There was a dose-dependent inhibition of tumor growth. Tumor size in mice treated with 15 mg/kg G4 antisense oligonucleotides was significantly smaller than in control groups, and represented 39% of control mean tumor size. In contrast, administration of G4 scrambled oligonucleotides (G4 SM) at 15 mg/kg provided no therapeutic activity. None of the mice treated with oligonucleotides displayed any signs of toxicities, and both doses of oligonucleotides were well tolerated. A dose of 15 mg/kg was selected for the future combination treatment regimens with anticancer drugs.

At paragraph 9, applicants disclose that to correlate G4 antisense oligonucleotide tumor growth inhibitory effects with XIAP protein expression, the changes in XIAP expression at the end of the *in vivo* treatment with 15 mg/kg of G4, F3, and C5 antisense and scrambled oligonucleotides were examined. At day 21 or 24 post-tumor inoculation when tumors reached 1 cm^3 in size, tumors were harvested and lysates from tumor homogenates were used for western blot analysis. XIAP and β-actin antibodies against the human protein were used, allowing for determination of human XIAP levels obtained from tumor cells specimens without contamination from XIAP derived from mouse cells. XIAP protein levels in tumors treated with G4 antisense oligonucleotides were quantitated by densitometry. Applicants found that XIAP protein levels were significantly reduced to approximately 85% of control tumors ($P < 0.005$) (Exhibit J-A and B). XIAP protein in tumors treated with G4 scrambled oligonucleotides were reduced in size by 24% of control tumors. These results indicated that inhibition of H460 tumor growth by G4 antisense oligonucleotides correlated with the down-regulation of XIAP protein expression.

In paragraph 10, applicants disclose that to evaluate whether XIAP antisense oligonucleotide administration results in direct tumor cell kill, the histology of tumors

after treatment was examined both for morphology and ubiquitin immunostaining (Exhibit K-A and B). At day 21 or 24 post-tumor inoculation, tumors treated with 15 mg/kg of G4 antisense, scrambled oligonucleotides, or saline control were excised, sectioned, and stained with hematoxylin and eosin. The results demonstrated that tumors in animals administered given XIAP antisense oligonucleotides treatment contained an increased number of dead cells, identified morphologically by their amorphous shape and condensed nuclear material.

Efficacy testing of combination therapy

At paragraph 12, applicants disclose the *in vivo* efficacy of combination therapy. Applicants combined treatments of G4 antisense oligonucleotides with a known chemotherapeutic agent. The therapeutic efficacy of vinorelbine, an agent used for lung cancer treatment, was assayed in the presence and absence of G4 antisense oligonucleotides or scrambled oligonucleotides. Treatment was initiated on day 3 after tumor inoculation. Exhibit L presents the *in vivo* efficacy results for 5 mg/kg and 10 mg/kg doses of vinorelbine (VNB) given to H460 tumor-bearing mice and compared with saline controls. Each of the two regimens induced significant tumor growth suppression in a dose-dependent manner without showing significant signs of undesirable toxicity (i.e., body weight loss). When administration of G4 antisense oligonucleotides (15 mg/kg) was combined with vinorelbine (5 mg/kg) for the treatment of H460 tumors, a more pronounced delay of H460 tumor growth was observed compared to either treatment administrated alone. Again, the mice did not show any significant signs of toxicity (i.e., body weight loss). Mean tumor size in mice treated with 5 mg/kg vinorelbine in the presence or absence of G4 AS or scrambled oligonucleotides (G4 SC) was compared on day 29 (Exhibit L-A and L-B). The tumor sizes in the group receiving combination therapy was 0.22 ± 0.03 cm^3, significantly smaller than the tumor sizes of groups receiving any other treatment (tumor size in control mice receiving 5 mg/kg vinorelbine alone or a combination of vinorelbine and G4 scrambled oligonucleotide was 0.59 ± 0.04 and 0.48 ± 0.05 cm^3, respectively).

In sum, using routine methods described in our specification at the time of filing, applicants have now demonstrated the *in vivo* therapeutic efficacy of antisense oligonucleotides for enhancing apoptosis in a cell of a mammal and for the treatment of a patient diagnosed as having a proliferative disease. Clearly, applicants have enabled a method for inducing apoptosis in a cell in a mammal and a method for treating a patient having a proliferative disease, the methods involving administering antisense nucleic acids complementary to XIAP (SEQ ID NO:3) to inhibit XIAP biological activity. Thus, the enablement rejection may be withdrawn.

Information Disclosure Statement

Applicant's note that the Examiner did not initial and return page 3 of form PTO 1449. Applicants hereby request that this statement be initialed and returned with the next Office Action.

CONCLUSION

Enclosed is a petition to extend the period for replying for three months, to and including date May 20, 2003.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: May 20, 2003

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